Mol Breeding (2015) 35:124
DOI 10.1007/s11032-015-0320-7

The gene *PmYB* confers broad-spectrum powdery mildew resistance in the multi-allelic *Pm2* chromosome region of the Chinese wheat cultivar YingBo 700

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Received: 17 February 2015 / Accepted: 5 May 2015 / Published online: 12 May 2015

Abstract It is important to detect wheat broad-spectrum powdery mildew resistance (*Pm*) genes in commercial cultivars with superior agronomic performance. YingBo 700, a Chinese commercial cultivar registered and released in 2012, showed a broad spectrum of resistance to 48 of 49 tested *Blumeria graminis* f. sp *tritici* (*Bgt*) isolates from different regions of China. Genetic analysis of F_2 populations from the crosses YingBo 700 × Mingxian 169 and YingBo 700 × Shimai 15 and $F_{2:3}$ lines from YingBo 700 × Mingxian 169 demonstrated that the resistance was controlled by a single dominant resistance gene, which was temporarily designated *PmYB*. Using molecular markers and bulked segregant analysis of the $F_{2:3}$ lines from YingBo 700 × Mingxian 169, *PmYB* was mapped to the multi-allelic *Pm2* region of chromosome arm 5DS co-segregated with the sequence-characterized amplified region (SCAR) marker *SCAR112* and flanked by the simple sequence repeat (SSR) marker *Cfd81* and SCAR marker *SCAR203* with genetic distance of 0.9 and 1.9 cM, respectively. YingBo 700 showed a unique response pattern to different *Bgt* isolates compared with the lines with documented *Pm2a*, *Pm2b*, *Pm48*, *PmLX66*, *PmW14*, *PmZ155* and *PmX3986-2* on chromosome arm 5DS. Therefore, *PmYB* appears to be a novel allele in this multi-allelic region. In order to validate applicability of the closely linked markers for marker-assisted selection, the closely linked markers *Cfd81*, *SCAR112*, *SCAR203* and *Mag6176* were evaluated for applicability in diagnosing the resistance gene in 62 cultivars from different regions of China. The results will contribute to rapidly transfer of *PmYB* into more cultivars.

Keywords Commercial cultivar · Powdery mildew · *Pm2* · MAS · *Triticum aestivum*

Abbreviations

Bgt	*Blumeria graminis* f. sp. *tritici*
BSA	Bulked segregant analysis
EST	Expressed sequence tag
IT	Infection type
MAS	Marker-assisted selection
Pm	Powdery mildew resistance

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Electronic supplementary material The online version of this article (doi:10.1007/s11032-015-0320-7) contains supplementary material, which is available to authorized users.

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SCAR Sequence-characterized amplified region
SSR Simple sequence repeat

Introduction

Common wheat (*Triticum aestivum* L.) is one of the most important food crops in the world, but its yield is continuously challenged by various wheat diseases (Costanzo and Bàrberi 2014). Worldwide wheat powdery mildew, caused by *Blumeria graminis* f. sp. *tritici* (*Bgt*), is a devastating and rapidly spreading disease that can cause severe yield losses in a short time (Hao et al. 2014). Of all the defensive measures, host resistance is the most effective, economical and environmentally sound and consistently used method (Petersen et al. 2014; Wang et al. 2015). In the history of breeding for host resistance, some powdery mildew resistance (*Pm*) genes have played a key role reducing the yield losses of wheat, such as *Pm8*, one of the most effective and widely popularized *Pm* gene in the last century that has made great contributions to the control of the wheat powdery mildew for more than two decades (Zeller and Hsam 1996; Huang and Röder 2004; An et al. 2013). Excessive use of this gene led to 'boom-bust' cycles of epidemics in the 1990s when new isolates appeared that were capable of overcoming *Pm8* (Ren et al. 1997; McIntosh et al. 2011; Ren et al. 2009). Currently, even the most effective and popular resistance gene *Pm21* is also suffering severe challenge, since some variant isolates are able to defeat this gene (Shi et al. 2009; Ren et al. 2011). Therefore, gene discovery and allele mining for powdery mildew resistance is a continuous challenge.

Up to now, about 70 formally designated *Pm* genes/alleles *Pm1–Pm54* at 49 loci (*Pm8* is allelic to *Pm17*, *Pm18* = *Pm1c*, *Pm22* = *Pm1e*, *Pm23* = *Pm4c* and *Pm31* = *Pm21*) and an additional about 20 temporarily named *Pm* genes have been identified on all the chromosomes except 3D in common wheat and its relatives (Hao et al. 2014; McIntosh et al. 2014). Of these genes, some have been not effective to the new virulent *Bgt* isolates, some are identified in host lines with linkage drags that lead to poor agricultural performance, so are seldom used in resistance breeding, and some need further time before their use (e.g., Zeller and Hsam 1996; Friebe et al. 1994; Ma et al. 2014). However, one class of *Pm* genes has been identified in commercial cultivars that provide broad-spectrum powdery mildew resistance (e.g., Huang et al. 2012; Zhao et al. 2013). A combination of excellent agronomic performance and broad-spectrum resistance offers an attractive prospect in resistance breeding. In China, some cultivars have been reported to show resistance to powdery mildew by a large number of powdery mildew isolates (Li et al. 2011; Song et al. 2012). On chromosome arm 2AL, two *Pm* genes *PmYm66* linked to *Ksum193* and *PmHNK54* flanked by *Barc5* and *Gwm312* were identified in Yumai 66 and Zheng 9754, respectively (Hu et al. 2008a; Xu et al. 2011). In Tangmai 4, the *Pm* gene *PmTm4* was flanked by *Gwm611* and *EST92* on chromosome arm 7BL (Hu et al. 2008b). Jimai 22 and Liangxing 99 both carry a single *Pm* gene on chromosome arm 2BL, which are linked to *Wmc149* and flanked by *BE604758* and *Gwm120*, respectively (Yin et al. 2009; Zhao et al. 2013). Three *Pm* genes *PmLX66*, *PmW14* and *PmZ155* on chromosome 5DS in Liangxing 66, Wennong 14 and Zhongmai 155, respectively, were responsible for their resistance to powdery mildew (Huang et al. 2012; Song et al. 2014; Sun et al. 2015). *PmHNK* on chromosome arm 3BL confers resistance to powdery mildew in Zhoumai 22 (Xu et al. 2010).

YingBo700 is a commercial winter wheat cultivar that was registered and released in Hebei Province of China in 2012 that has superior yield performance and excellent powdery mildew resistance. To identify the resistance gene(s) in YingBo 700 and use it in resistance breeding, the following researches were carried out: (1) examine the inheritance of the resistance to powdery mildew; (2) determine the chromosome location of the resistance gene(s) using molecular markers; (3) test the seedling reaction of YingBo 700 to a collection of *Bgt* isolates from different regions of China and compare its response pattern with the documented *Pm* genes near it; and (4) validate the applicability of the closely linked markers for use in marker-assisted selection (MAS) in different wheat genetic backgrounds.

Materials and methods

Plant materials

YingBo 700 is a winter wheat cultivar developed from the cross of Taigu genetically male-sterile wheat and

the wheat line Ji935031 by the Hebei YingBo Seed Technology Co. Ltd., Xingtai City, Hebei Province, China (Zhang et al. 2012). Two susceptible cultivars Mingxian 169 and Shimai 15 were crossed with YingBo 700 to produce F_1 hybrids, F_2 populations and $F_{2:3}$ lines for genetic analysis and molecular mapping of the *Pm* gene(s) in YingBo 700. Wheat lines or cultivars Ulka/8*Cc (with *Pm2a*), KM2939 (with *Pm2b*), Liangxing66 (with *PmLX66*), Tabasco (with *Pm48*), Wennong14 (with *PmW14*), X3986-2 (with *PmX3986-2*) and Zhongmai155 (with *PmZ155*) that carry documented *Pm* genes were used to compare their *Pm* genes with that of YingBo 700. Wheat cultivars Mingxian 169, Shimai 15 and Huixianhong were used as susceptible controls. A set of 62 wheat commercial cultivars and important breeding lines (Supplementary Table S1) that are widely grown or used in China were selected to survey the applicability of the closely linked markers on chromosome arm 5DS for MAS in different genetic backgrounds.

Disease assessment at the seedling stage

Forty-nine single-spore *Bgt* isolates with different virulence pattern were collected from different wheat production regions (Supplementary Table S2). They were used to determine the reaction patterns of YingBo 700 and wheat genotypes with documented *Pm* genes in this study. *Bgt* isolate B03, which is avirulent on YingBo 700 and virulent on Mingxian 169 and Shimai 15, was selected to inoculate YingBo 700, two susceptible parents Mingxian 169 and Shimai 15 and the derived F_1 hybrids, F_2 populations from YingBo 700 × Mingxian 169 and YingBo 700 × Shimai 15 and the $F_{2:3}$ lines of YingBo 700 × Mingxian 169 for genetic analysis and mapping of the gene(s) conferring powdery mildew resistance in YingBo 700.

Evaluation of seedling reactions to different *Bgt* isolates was carried out in a humidity environment at 18/12 °C (day/night) with a photoperiod of 12–14 h of light per day (Si et al. 1992). Each isolate was placed in an independent and enclosed space to avoid cross-infection between different isolates. When the first leaf was fully unfolded, plants of the susceptible control Mingxian 169 with newly increased conidia were dusted evenly. The infection type (IT) of each plant was scored based on the 0–4 scale when the pustules were fully developed on the first leaf of susceptible controls at about 14–15 days after inoculation. Based on IT, the plants were classified as a resistant (R, ITs 0–2) or a susceptible phenotype (S, ITs 3–4) to powdery mildew (Si et al. 1992).

Marker analysis

The total genomic DNA of YingBo 700, Mingxian 169 and the $F_{2:3}$ lines from the cross YingBo 700 × Mingxian 169 was extracted after evaluation of their IT scores using phenol/chloroform method (Sharp et al. 1988). Equal amounts of genomic DNA of 10 resistant (with IT of 0) and 10 susceptible (with IT of 4) F_2 plants were pooled for bulked segregant analysis (BSA) and compared to the banding patterns of resistant and susceptible parents (Michelmore et al. 1991). Fifty molecular markers linked to the documented *Pm* genes and about 100 simple sequence repeat (SSR), expressed sequence tag (EST) and sequence-characterized amplified region (SCAR) markers that were distributed on chromosomes 4DL, 5DS and 7BS, were used to test for polymorphisms between the parents and the bulks. PCR performance and resolution and visualization of the PCR products were conducted following the methods of An et al. (2013). Markers showing polymorphisms between the bulks and the parents were tested against the $F_{2:3}$ lines of YingBo 700 × Mingxian 169 to map the gene conferring powdery mildew resistance in YingBo 700.

Statistical analysis

Deviation of observed phenotypic data from theoretically expected segregation ratios was evaluated by Chi-square (χ^2) test for goodness of fit. Software MAPMAKER 3.0 was used to construct linkage map for molecular markers and the resistance gene locus. A LOD threshold value 3.0 and a maximum map distance 37.5 cM were used to declare a linkage group. (Lincoln et al. 1992). Map distance was calculated from the recombination values using the Kosambi function (Kosambi 1944).

Validation of the applicability of the closely linked markers for marker-assisted selection (MAS)

Sixty-two cultivars or important breeding lines from different wheat production regions of China were used to validate the closely linked markers in different backgrounds for MAS (Supplementary Table S1).

Results

Inheritance of powdery mildew resistance in YingBo 700

YingBo 700 produced an immune reaction with IT 0 when inoculated with *Bgt* isolate B03, whereas Mingxian 169 and Shimai 15 were both highly susceptible with IT 4. YingBo 700 was crossed with Mingxian 169 and Shimai 15 to produce F_1 and F_2 populations. The F_1 generations of YingBo 700 × Mingxian 169 and YingBo 700 × Shimai 15 were both resistant to *Bgt* isolate B03 with IT 0, indicating the dominant nature of the powdery mildew resistance in YingBo 700. The F_2 populations of YingBo 700 × Mingxian 169 and YingBo 700 × Shimai 15 segregated 112 resistant to 32 susceptible plants ($\chi^2_{3:1} = 0.45$, $df = 1$, $P = 0.50$) and 163 to 63 ($\chi^2_{3:1} = 1.00$, $df = 1$, $P = 0.32$), respectively, both fitting a single dominant gene segregation ratio. In order to confirm these results, F_2 population of YingBo 700 and Mingxian 169 were transplanted to the field after evaluating the IT of each F_2 plant, and 115 plants survived to produce F_3 seeds. Thirty plants of each $F_{2:3}$ line were tested for their powdery mildew response to the *Bgt* isolate B03. The $F_{2:3}$ population segregated as 27 homozygous resistant, 57 segregating and 31 homozygous susceptible, fitting a 1:2:1 segregation ratio ($\chi^2 = 0.29$, $df = 2$, $P = 0.87$). Therefore, it was concluded that powdery mildew resistance to *Bgt* isolate B03 in YingBo700 was controlled by a single dominant gene, which was designated *PmYB*.

To determine consistency of the resistance of *PmYB* to other *Bgt* isolates and the transfer to its progenies, all other ten isolates (B05, B08, B15, B16, B28, B30, B33, B39, B41 and B50) that were avirulent on YingBo 700 and virulent on Mingxian 169 were selected to inoculate five randomly selected $F_{2:3}$ lines with homozygous resistant (No.21, 28, 32, 69 and 84) and five with segregating phenotype (No.4, 16, 24, 34 and 37) selected by B03, respectively. The results demonstrated that all the five $F_{2:3}$ lines were homozygously resistant to all the ten *Bgt* isolates, and the other five $F_{2:3}$ lines had a segregating phenotype to all of the ten *Bgt* isolates. Therefore, *PmYB* confers consistent resistance to different *Bgt* isolates, and the resistance could be transferred to its progenies.

Molecular mapping of *PmYB*

To determine the genetic location of *PmYB*, the F_2 and $F_{2:3}$ progenies from the cross YingBo 700 to the susceptible cultivar Mingxian 169 were used for BSA. Fifty molecular markers linked to the known *Pm* genes were first screened for the occurrence of polymorphisms between the parents as well as the resistant and susceptible DNA bulks. *Pm2*-linked SSR marker *Cfd81* showed a polymorphism between the parents and bulks. *Cfd81* was further used to genotype the $F_{2:3}$ lines of YingBo 700 × Mingxian 169 and was mapped 0.9 cM away from *PmYB*. Since *Cfd81* has been mapped to three loci on chromosome arms 4DL, 5DS and 7BS, 13, 32 and 32 SSR markers were subsequently examined for polymorphisms between parents and bulks. Only *Cfd40*, *Cfd78* and *Gwm159* which are located on chromosome arm 5DS showed polymorphisms between the parents and bulks, and all the markers on chromosome arms 4DL and 7BS showed no polymorphisms. In order to increase marker density around *PmYB* locus of the chromosome arm 5DS, a total of 25 EST-derived markers and two SCAR markers *SCAR112* and *SCAR203* that are located on chromosome arm 5DS were tested for polymorphisms. The EST marker *Mag6176* linked to the documented *Pm* gene *PmD57-5D* and two SCAR markers *SCAR112* and *SCAR203* revealed polymorphisms between parents and bulks. Then, *Cfd40*, *Cfd78*, *Gwm159*, *SCAR112*, *SCAR203* and *Mag6176* were used to genotype the $F_{2:3}$ lines. *PmYB* was linked to these markers at a genetic distance of 13.5, 10.2, 4.8, 0, 1.9 and 2.8 cM, respectively. The loci order of the linked markers was *Xcfd78–Xmag6176–Xscar203–PmYB/SCAR112–Xcfd81–Xgwm159–Xcfd40* (Fig. 1). Previous studies showed that *Cfd81*, *SCAR112*, *SCAR203* and *Mag6176* loci on chromosome arm 5DS were all located in the deletion bin 5DS–1–0–0.63 (Ma et al. 1994; Gao et al. 2012; Huang et al. 2012). Therefore, *PmYB* was delineated in the same physical chromosome bin.

Comparison of *PmYB* and the documented *Pm* genes on chromosome arm 5DS

Forty-nine *Bgt* isolates with different virulence were used to test the reaction patterns of YingBo 700 and documented resistant stocks with specific *Pm* genes on chromosome arm 5DS (Fig. 2; Supplementary

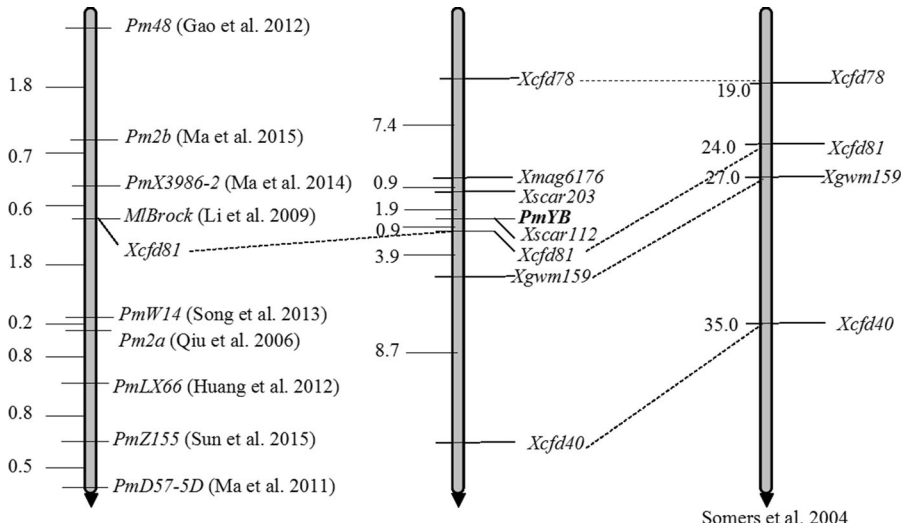

Fig. 1 Genetic linkage maps of powdery mildew resistance gene *PmYB* using the $F_{2:3}$ lines of YingBo 700 × Mingxian 169 and its locus comparison with the documented *Pm* genes on chromosome arm 5DS using the marker locus *Xcfd81* and the linkage map of linked SSR markers by Somers et al. (2004). Black arrows point to the centromere direction. Genetic distance was shown to the left of the map in cM

Table S2). YingBo 700 showed resistance to 48 of the 49 *Bgt* isolates, with isolate B56 (with IT 4) being the only exception. Ulka/8*Cc (*Pm2a*), KM2939 (*Pm2b*) and Tabasco (*Pm48*) were all resistant to isolate B56, but susceptible to 13 other isolates (B07, B08, B10, B16, B28, B29, B30, B32, B38, B39, B40, B50 and B51), two isolates (B38 and B40) and two isolates (B29 and B38), respectively. Four other resistant stocks Liangxing 66 (with *PmLX66*), Wennong 14 (with *PmW14*), X3986-2 (with *PmX3986-2*) and Zhongmai 155 (with *PmZ155*) with tentatively designated *Pm* genes were also tested for their reaction patterns to these 49 *Bgt* isolates. They were susceptible to 11 isolates (B07, B28, B29, B30, B32, B38, B40, B50, B51, B69 and B80), three isolates (B28, B29 and B38), 17 isolates (B04, B09, B28, B29, B32, B33, B38, B40, B50, B62, B65, B69, B71, B77, B80, B82 and B83) and two isolates (B38 and B81), respectively. Therefore, YingBo 700 showed a significantly different reaction pattern from those of Ulka/8*Cc, KM2939, Tabasco, Liangxing 66, Wennong 14, X3986-2 and Zhongmai 155 in 14, 3, 3, 12, 4, 18 and 3 of 49 isolates, respectively. Therefore, *PmYB* is different from the documented *Pm2a*, *Pm2b*,

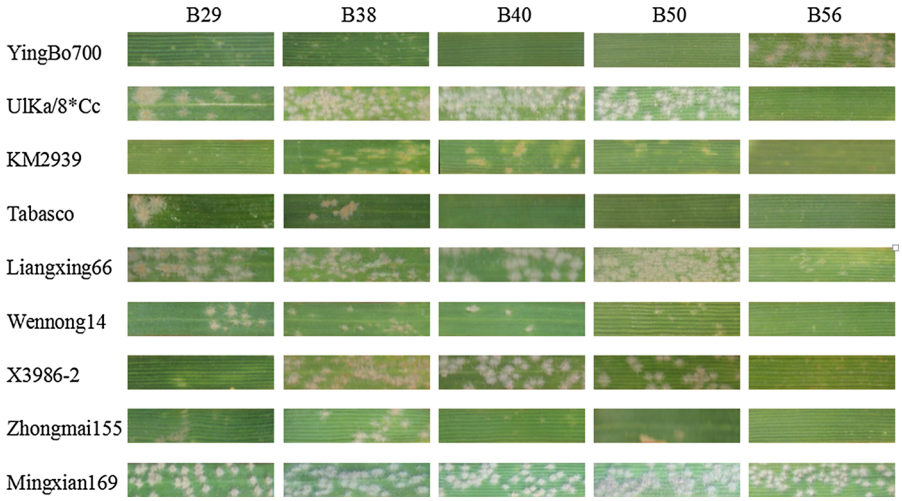

Fig. 2 Reactions of YingBo 700 and the documented wheat lines Ulka/8*Cc (*Pm2a*), KM2939 (*Pm2b*), Tabasco (*Pm48*), Liangxiang 66 (*PmLX66*), Wennong 14 (*PmW14*), X3986-2 (*PmX3986-2*) and Zhongmai 155 (*PmZ155*) to several selected *Blumeria graminis* f. sp. *tritici* (*Bgt*) isolates selected from 49 *Bgt* isolates in this study

Pm48, *PmLX66*, *PmW14*, *PmX3986-2* and *PmZ155* on chromosome arm 5DS.

In order to further compare *PmYB*-linked markers and their amplification patterns with those of documented *Pm* genes on chromosome arm 5DS, the closely linked markers *Cfd81*, *SCAR203*, *SCAR112* and *Mag6176* were used to evaluate the documented resistant stocks with *Pm2a*, *Pm2b*, *Pm48*, *PmLX66*, *PmW14*, *PmX3986-2* and *PmZ155*. Primer pairs CFD81 amplified a 255 bp polymorphic band which was linked to *PmYB* in the multi-allelic *Pm2* chromosome region in YingBo 700 and other documented resistant stocks carrying *Pm2a*, *Pm2b*, *Pm48*, *PmLX66*, *PmW14*, *PmX3986-2* and *PmZ155*, while in the susceptible parent, it produced a 260 bp band (Fig. 3). The three primer pairs SCAR112, SCAR203 and MAG6176 amplified 200, 120 and 550 bp polymorphic bands, while in susceptible parents, there were no band and 202 and 600 bp bands, respectively. Therefore, the amplification patterns of the closely linked markers were all same in YingBo 700 and other documented resistant stocks with *Pm2a*, *Pm2b*, *Pm48*, *PmLX66*, *PmW14*, *PmX3986-2* and *PmZ155*.

Validating applicability of the linked markers for marker-assisted selection

From the markers comparison, it was concluded that the closely linked markers could not distinguish *PmYB* in the wheat phenotypes with *Pm* genes in the multi-allelic *Pm2* chromosome region. In order to survey the applicability of the closely linked markers in other genomic backgrounds, 62 common wheat cultivars or important breeding lines with no documented *Pm* genes in the multi-allelic *Pm2* chromosome region were analyzed using four closely linked markers namely *Cfd81*, *SCAR112*, *SCAR203* and *Mag6176* (Supplementary Table S1; Fig. 3). Primer pairs CFD81 amplified *PmYB*-linked polymorphic band with 255 bp in YingBo 700 and two other wheat cultivars Jimai 22 and Liangxing 99, and in other 60 cultivars it amplified the related bands ranging from 258 to 262 bp. These results implied that *Cfd81* could detect *PmYB* in 60 of 62 cultivar or breeding line backgrounds with no documented *Pm* genes in the multi-allelic *Pm2* chromosome region, except for Liangxing 99 and Jimai 22. The three primer pairs SCAR112, SCAR203 and MAG6176 was also detected *PmYB* in 60 of 62 cultivar or breeding line backgrounds except for Liangxing 99 and Jimai 22, which was the same as CFD81. These results indicated that these closely linked markers may be used in detecting *PmYB* in most of the genomic backgrounds with no documented *Pm* genes in the multi-allelic *Pm2* chromosome region except for Liangxing 99 and Jimai 22 when *PmYB* was transferred into these cultivars through conventional hybridization.

Discussion

YingBo 700 is a commercial wheat cultivar registered and released in Hebei province, China. With its

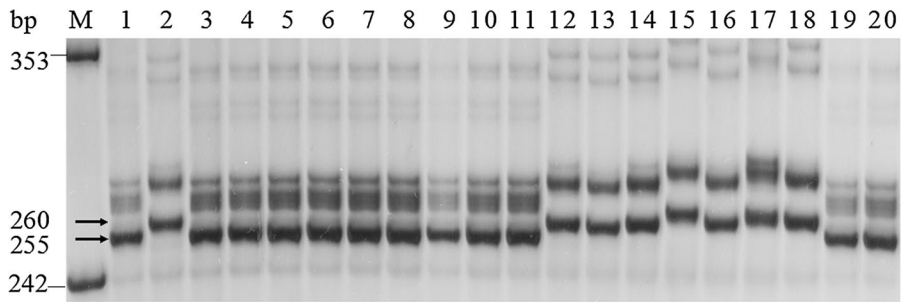

Fig. 3 PCR amplification patterns of YingBo 700, wheat genotypes with documented *Pm* genes in multi-allelic *Pm2* chromosome region and some wheat cultivars or important breeding line using *PmYB*-linked markers *Cfd81*. M is DNA marker pUC18 *Msp* I; Lanes 1–2 is YingBo 700 and Mingxian 169; Lanes 3–11 is documented resistant stocks with sequential order of UlKa/8*Cc (*Pm2a*), KM2939 (*Pm2b*), Tabasco (*Pm48*), Liangxiang 66 (*PmLX66*), Wennong 14 (*PmW14*), X3986-2 (*PmX3986-2*), Zhongmai 155 (*PmZ155*), Brock (*MlBrock*) and D57-5D (*PmD57-5D*); Lanes 12–20 are some wheat cultivars or important breeding line with sequential order of Shixin 828, Kenong 9204, Shimai 15, Lumai 21, Yangmai 158, Zhengmai 366, Zhou 8425B, Liangxing 99 and Jimai 22. The arrows indicate 255 and 260 bp polymorphic bands in YingBo 700 and Mingxian 169, respectively

superior agronomic performance, cold hardiness and resistance to several main wheat diseases, such as wheat powdery mildew, stem rust (caused by *Puccinia triticina* Eriks) and stripe rust (caused by *P. striiformis* f. sp. *tritici* Eriks), YingBo 700 is preferable and has attracted the attention of farmers and breeders quickly since it released in 2012. In this study, the powdery mildew resistance in YingBo 700 was shown to be inherited as a monogenic trait. A single dominant gene with resistance to different *Bgt* isolates, tentatively designated *PmYB*, was assigned *Pm2* locus on chromosome arm 5DS with linked marker loci consistent with the reported linkage map by Somers et al. (2004) (Fig. 1).

In the multi-allelic *Pm2* chromosome region, several *Pm* genes have been reported. *Pm2a* was the first *Pm* gene reported on chromosome arm 5DS with an original designation *Pm2* and a re-designation *Pm2a* when the new allele *Pm2b* in the same locus was discovered (McIntosh and Baker 1970; Ma et al. 2015). Gene *Pm2a* originated from the common wheat cultivar Ulka and was firstly located on chromosome 5D by monosomic analysis (McIntosh and Baker 1970). Then, *Pm2a* was mapped to chromosome arm 5DS and linked to the RFLP marker *BCD1871* at a genetic distance 3.5 cM by Ma et al. (1994) and SSR marker *Cfd81* with genetic distance of 2.0 cM by Qiu et al. (2006) in the near-isogenic line UlKa/8*Cc developed from Ulka and its recurrent parent Chanceller(Cc) (Briggle 1969) (Fig. 1). Two other *Pm* genes *PmD57-5D* and *MlBrock* were also mapped at the same locus as *Pm2a* in the two wheat lines D57 and Brock. Based on their genetic position and their reaction patterns to different *Bgt* isolates, *PmD57-5D* and *MlBrock* were both considered to be *Pm2a* (Li et al. 2009; Ma et al. 2011). Over a considerable period of time to the present, *Pm2a* has been widely used in resistance breeding and remains effective against most of the *Bgt* isolates in China (Ma et al. 2014). However, more and more *Bgt* isolates have defeated *Pm2a* in recent years due to the race-specific nature of resistance and its excessive deployment as a single resistance gene (Ma et al. 2014). In this study, *Pm2a* was susceptible to 13 of the 49 *Bgt* isolates tested. *PmYB* showed significantly broader resistant spectrum with only one virulent isolate (i.e., B56). Therefore, *PmYB* is significantly different from *Pm2a* and has much broader resistant spectrum than *Pm2a*.

Pm2b, the second allele of *Pm2*, was recently identified in a putatively *Agropyron cristatum*-derived breeding line KM2939 (Ma et al. 2015). It also showed a broad resistant spectrum with resistance to 47 of 49 *Bgt* isolates susceptible to B38 and B40. *PmYB* was resistant to *Bgt* isolate B38 and B40 but susceptible to B56, showing the reverse response of *Pm2b* to these three isolates. Also, the IT of *PmYB* to B28 and B30 were both 0, while *Pm2b* had a type 2 IT to both isolates. The third difference is the reaction patterns of the resistance phenotype. The most common resistance phenotype of *Pm2b* to the *Bgt* isolates (32 of 48) was a hypersensitive response with an IT of 0; while *PmYB* did not produce a hypersensitive response phenotype to the isolates in total. Therefore, *PmYB* is also significantly different from *Pm2b*.

The gene *Pm48* was another *Pm* gene also located in the multi-allelic *Pm2* chromosome region in the German cultivar Tabasco (Gao et al. 2012). It was firstly temporarily designated as *Pm46* and then formally re-designated *Pm48* (McIntosh et al. 2014). Allelism test between *Pm48* and *Pm2a* using a F_2 population of 536 plants showed that *Pm48* and *Pm2a* were closely linked to each other, but were not allelic (Gao et al. 2012). In this study, *PmYB* was separated by a genetic distance of 2.0 cM from *Pm48* and therefore needs to be distinguished from *Pm48* (Fig. 1). Disease spectrum analysis indicated that *Pm48* was susceptible to B29 and B38 in the 49 *Bgt* isolates tested, while *PmYB* was resistant to B29 and B38. Meanwhile, the phenotype of *Pm48* to B28 was moderate resistant with an IT of 2, while *PmYB* was immune to this isolate. Therefore, *PmYB* is also different from *Pm48*.

Four temporarily designated *Pm* genes (i.e., *PmLX66*, *PmW14*, *PmZ155* and *PmX3986-2*) were also assigned to chromosome arm 5DS in the three Chinese wheat cultivars Liangxing 66, Wennong 14 and Zhongmai 155 and the Chinese wheat line X3986-2, respectively (Huang et al. 2012; Ma et al. 2014; Song et al. 2014; Sun et al. 2015). Of these four genes, *PmLX66* and *PmW14* were proven to be alleles of *Pm2* (Sun et al. 2015), and *PmX3986-2* and *PmZ155* were located at a 1 cM from *Pm2a*, so their allelic relationships need to be further confirmed. Although *PmLX66*, *PmZ155* and *PmW14* were also derived from commercial cultivars, they have a narrower resistant spectrum than *PmYB*, with 11, 3 and 2 virulent *Bgt* isolates, respectively. *PmX3986-2* showed even

narrower resistant spectrum with resistance frequency of only 65.3 %. Therefore, *PmYB* is also different from *PmLX66*, *PmW14*, *PmZ155* and *PmX3986-2* and shows broader resistant spectrum than these four genes.

From the comparison of the resistant spectrum between *PmYB* and the documented *Pm* genes on chromosome arm 5DS, *PmYB* has a broader resistant spectrum, which is different from the documented genes *Pm2a*, *Pm2b*, *Pm48*, *PmLX66*, *PmW14*, *PmZ155* and *PmX3986-2*. However, based on the linkage maps, *PmYB* shares the same linked markers and similar locus with these documented *Pm* genes (Fig. 1). Amplification patterns of four closely linked markers also were all the same between YingBo 700 and the documented resistant stocks, which further demonstrated that *PmYB* shared the same locus with the documented *Pm* genes (Fig. 3). Therefore, *PmYB* is most likely a novel allele in the multi-allelic *Pm2* chromosome region. An allelism test is still required to confirm the allelic relationship between *PmYB* and the documented genes in the chromosome region.

In this study, *PmYB* was mapped to a hotspot for resistance alleles, which may be easily contributed to its use for resistance breeding. Other hotspots for resistance to powdery mildew include the *Pm1a–1e* locus on chromosome 7AL (Hsam et al. 1998; Singrün et al. 2003), *Pm3a–3j* locus on chromosome 1AS (Zeller and Hsam 1998), *Pm4a–4d* locus on chromosome 2AL (Schmolke et al. 2012), *Pm5a–5e* locus on chromosome 7BL (Hsam et al. 2001; Huang et al. 2000a, 2003) and *Pm24a–24b* locus on chromosome 1DS (Huang et al. 2000b; Xue et al. 2012). More importantly, *PmYB* originates from a common wheat cultivar associated with superior agronomic performance, which is preferable to breeders unlike genes from wild-related species or lines with poor agronomic performance. The identification of *PmYB* and its closely linked molecular markers will facilitate the use of *PmYB* in wheat improvement against powdery mildew. In fact, four closely linked markers of *PmYB* were validated following their use in MAS of 62 wheat cultivars or important breeding line from different regions of China. If *PmYB* were transferred to these cultivars through hybridization, *PmYB* can be detected in most genomic backgrounds tested in this study combined with the four markers, apart from the documented resistant stocks with *Pm* genes in the multi-allelic *Pm2* chromosome region and two cultivars Liangxing 99 and Jimai 22 (Fig. 3; Supplement Table S1). Because of the similar locus, *PmYB* cannot be detected in these genomic backgrounds and also pyramided with these documented *Pm* genes. Liangxing 99 and Jimai 22 have single dominant *Pm* genes *Pm52* and *PmJM22* both on chromosome arm 2BL (Yin et al. 2009; Zhao et al. 2013). Although *PmYB* is located on a significantly different genetic locus from those of *Pm52* and *PmJM22*, the amplification pattern by the four closely linked markers of *PmYB* were same as Liangxing 99 and Jimai 22. This may be related to mutation of this locus that results in the change of base composition and number, but not producing resistance, or other known reasons. Therefore, *PmYB* cannot be distinguished in Liangxing 99 and Jimai 22 genomic backgrounds by the molecular markers. Apart from closely linked markers, other means were also used to detect *PmYB* in Liangxing 99 and Jimai 22 backgrounds. Disease spectrum analysis showed that *PmYB* had a significantly different reaction pattern from those of *Pm52* and *PmJM22* (Supplement Table S2), which indicated that resistant spectrum may be another mean to distinguish *PmYB* in Liangxing 99 and Jimai 22 backgrounds.

Acknowledgments This research was financially supported by the National High-Tech Research and Development Program of China No. 2011AA1001, the National Natural Science Foundation of China No. 31171550, the National Scientific and Technological Supporting Program of China No. 2013BAD01B02 and the Chinese Academy of Sciences No. XDA08030107.

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